Towards improved oncological treatment of esophageal and gastric cancer. Clinical and translational studies.

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Towards improved oncological treatment of esophageal and gastric cancer

Clinical and translational studies

DAVID BORG
FACULTY OF MEDICINE | LUND UNIVERSITY

I am a medical oncologist at the Skåne University Hospital in Lund. My clinical area of expertise is gastrointestinal cancers. I have a particular interest in esophageal and gastric cancers and the aim of this thesis was to improve the oncological treatment strategies in these diseases.

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Towards improved oncological treatment of esophageal and gastric cancer

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Towards improved oncological treatment of esophageal and gastric cancer

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Abstract

Background

The overall aim of this thesis was to improve the oncological treatment strategies in esophageal and gastric adenocarcinoma (EGAC). Podocalyxin-like protein 1 (PODXL) has been associated with poor prognosis in other cancers and we wanted to investigate its potential role as a prognostic and predictive biomarker in resectable EGAC. Another aim was to assess how dose reductions and treatment delays of neoadjuvant chemotherapy affect outcome. Lastly we aimed to explore a novel palliative treatment strategy in esophageal adenocarcinoma with the primary objective to achieve durable improvement of dysphagia.

Methods

Expression of PODXL was assessed using immunohistochemistry on material from two patient cohorts with EGAC: one with 174 patients treated with surgery up-front and the other with 148 patients treated with neoadjuvant chemotherapy ± adjuvant chemotherapy.

For 63 patients in the neoadjuvant cohort, treated with EOX (epirubicin, oxaliplatin and capecitabine) followed by resection, we calculated the ratio of actual to planned cumulative dose and the ratio of planned to actual total duration and then associations of these measures with histopathologic response were assessed.

In the phase II PALAESTRA trial the treatment consisted of external beam radiotherapy with 20 Gy in five fractions followed by four cycles of chemotherapy.

Results

In paper I we show that PODXL expression is an independent prognostic biomarker in EGAC and the results in paper II indicate that PODXL expression is predictive for benefit of neoadjuvant ± adjuvant chemotherapy. In paper III it is suggested that treatment delays of neoadjuvant chemotherapy should be avoided in order to achieve a major histopathologic response. In the PALAESTRA study (paper IV) with 29 patients enrolled, 79% experienced improvement of dysphagia and for these the median duration of improvement was 12 months.

Conclusions

Promising steps have been taken towards improved treatment strategies but confirmation in additional studies is warranted.

Key words

Esophageal cancer, gastric cancer, adenocarcinoma, PODXL, biomarker, prognosis, prediction, chemotherapy, dose intensity, dysphagia, radiotherapy

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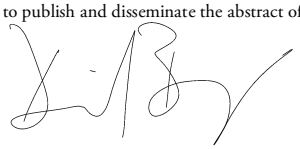
Signature  Date 2019-11-11

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To past, present and future patients

Contents

Thesis at a glance

Paper	Aims	Patients	Methods	Findings
I	To investigate the potential prognostic impact of PODXL expression in resected esophageal and gastric adenocarcinoma (EGAC)	174 patients with EGAC treated 2006-2010 with surgical resection up-front (cohort 1)	Retrospective Tissue microarrays Immunohistochemistry Survival analyses	PODXL expression was an independent prognostic biomarker for reduced time to recurrence and short overall survival
II	To assess if PODXL expression could be predictive of benefit from neoadjuvant ± adjuvant chemotherapy in EGAC	148 patients with resectable EGAC who started neoadjuvant chemotherapy 2008-2014 (cohort 2) Merger of cohort 1 and cohort 2	Retrospective Tissue microarrays or full face sections Immunohistochemistry Histopathologic response Survival analyses	PODXL expression in pre-treatment biopsies was an independent predictive biomarker for benefit of neoadjuvant ± adjuvant chemotherapy
III	To assess how dose reductions and treatment delays affect histopathologic response in patients with EGAC treated with neoadjuvant EOX	63 patients from cohort 2 who were treated with neoadjuvant EOX followed by surgical resection and for whom we hade detailed data of chemotherapy delivery	Retrospective Relative dose intensity, dose index, time index Histopathologic response	Avoidance of treatment delays (but not of dose reductions) of neoadjuvant EOX was associated with a major histopathologic response
IV	To investigate a novel treatment strategy in patients with incurable esophageal adenocarcinoma with the primary aim to achieve long-term improvement of dysphagia	29 patients with treatment-naîve esophageal adenocarcinoma, not eligible for curative treatment (cohort 3)	Phase II trial Short-course radiotherapy, 5 x 4 Gy, followed by chemotherapy (FOLFOX) Dysphagia assessment Survival analyses	The overall rate of dysphagia improvement was 79%, the median duration of improvement was 6.7 months for all patients and 12.2 months for the responders

List of papers

Papers included in the thesis

I. **Borg D**, Hedner C, Nodin B, Larsson A, Johnsson A, Eberhard J, Jirström K. Expression of podocalyxin-like protein is an independent prognostic biomarker in resected esophageal and gastric adenocarcinoma. *BMC Clin Pathol*. 2016;16:13. doi:10.1186/s12907-016-0034-8.

II. **Borg D**, Larsson AH, Hedner C, Nodin B, Johnsson A, Jirström K. Podocalyxin-like protein as a predictive biomarker for benefit of neoadjuvant chemotherapy in resectable gastric and esophageal adenocarcinoma. *J Transl Med*. 2018;16(1):290. doi:10.1186/s12967-018-1668-3.

III. **Borg D**, Hedner C, Jirström K, Johnsson A. Impact of dose reduction and treatment delay of neoadjuvant chemotherapy in gastric and esophageal adenocarcinoma. *(Manuscript)*

IV. **Borg D**, Sundberg J, Brun E, Kjellén E, Petersson K, Hermansson M, Johansson J, Eberhard J, Johnsson A. Palliative short-course hypofractionated radiotherapy followed by chemotherapy in esophageal adenocarcinoma: the phase II PALAESTRA trial. *Acta Oncol*. September 2019:1-7. doi:10.1080/0284186X.2019.1670861.

Papers not included in the thesis

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Abbreviations

ASR	Age-standardized incidence rate
CAPOX	Capecitabine, oxaliplatin
CI	Confidence interval
CT	Computed tomography
DI	Dose index
dMMR	Deficient mismatch-repair
DNA	Deoxyribonucleic acid
EBRT	External beam radiotherapy
EBV	Epstein-Barr virus
ECF	Epirubicin, cisplatin, fluorouracil
ECX	Epirubicin, cisplatin, capecitabine
EGAC	Esophageal and gastric adenocarcinoma
EMT	Epithelial-mesenchymal transition
EOX	Epirubicin, oxaliplatin, capecitabine
FDG	^{18}F-fluorodeoxyglucose
FLOT	Fluorouracil, calcium folinate, oxaliplatin, docetaxel
FOLFOX	Fluorouracil, calcium folinate, oxaliplatin
GE	Gastroesophageal
Gy	Gray
HER2	Human epidermal growth factor receptor 2
HR	Hazard ratio
IHC	Immunohistochemistry
ITT	Intention to treat
MSI	Microsatellite instability
OR	Odds ratio
OS	Overall survival
PD-L1	Programmed death-ligand 1

PET	Positron emission tomography
PODXL	Podocalyxin-like protein 1
PP	Per protocol
RDI	Relative dose intensity
RNA	Ribonucleic acid
S-1	Tegafur/gimeracil/oteracil
TCGA	The Cancer Genome Atlas
TI	Time index
TMA	Tissue microarray
TTR	Time to recurrence
SEMS	Self-expanding metal stent
VEGFR2	Vascular endothelial growth factor receptor 2
WHO	World Health Organization

Introduction to esophageal and gastric cancer

Anatomy

An overview of the anatomy is depicted in Figure 1. The cervical (upper) part of the esophagus begins below the hypopharynx (laryngopharynx) and stretches approximately 25 centimeters down the thoracic cavity, through the diaphragm where it connects to the stomach. The anatomical junction between the esophagus and stomach is called the true cardia and the transition from squamous cell epithelium to gastric mucosa is called the Z-line. Tumors arising in the cardia or the nearby area, i.e. the gastroesophageal (GE) junction is classified according to the modified Siewert classification [1, 2] in which type 1 are distal esophageal cancers, type II are true cardia cancers, and type III are subcardial cancers (of the stomach), Figure 2. The top of the stomach is called fundus, the body is called corpus and the distal part, before the duodenum, is called pylorus or antrum ventriculi.

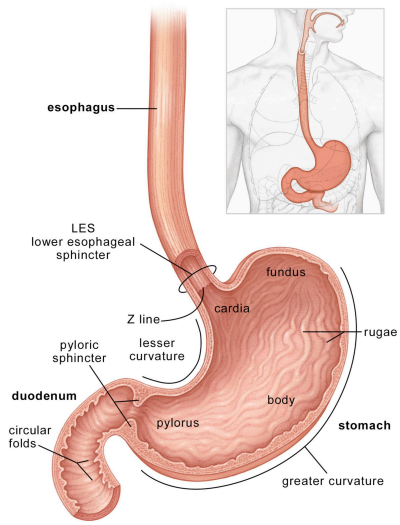

Figure 1.
Anatomy of the esophagus and stomach. Reprinted with permission from © 2017 Medical Art Library.

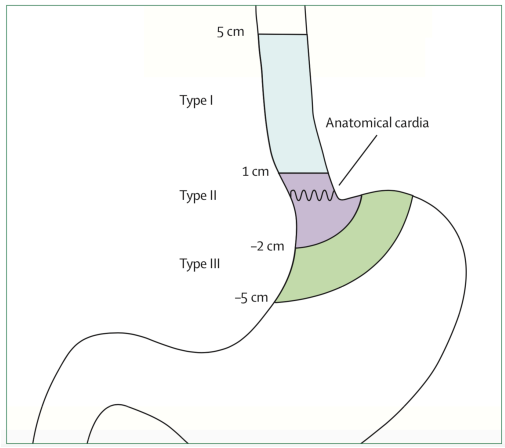

Figure 2.
Modified Siewert classification of tumors arising in the GE junction. Reprinted from Mariette et al. [2] with permission from Elsevier.

Epidemiology

In 2018 esophageal and gastric cancer were together the 4th most common malignancy in terms of worldwide incidence with an estimated number of 1.6 million new cases (age-standardized incidence rate (ASR) 17.4/100,000), with a male predominance. The number of deaths with approximately 1.3 million casualties was only superceded by that of lung cancer [3]. The exact proportion of gastric vs. esophageal cancer is somewhat difficult to assess due to the gradual shift in classification of tumors arising in the GE junction from gastric to esophageal cancer. The incidence of gastric cancer (including the GE junction) and esophageal cancer in 2018 was 1,030,000 and 570,000 new cases, respectively. Of note, if GE junction cancer was to be classified as esophageal cancer approximately one quarter [4] of the aforementioned number of gastric cancer cases might instead be classified as esophageal cancer.

The global incidence of gastric cancer has declined during the last five decades [5] and the highest incidence is seen in Asia followed by Eastern Europe and South America [4], Figure 3A. For esophageal cancer the incidence is highest in Eastern and Southeast Asia followed by Eastern and Southern Africa [6]. The most common esophageal subtype (almost 90%) worldwide is squamous cell carcinoma but in many Western countries, including Sweden, the incidence of adenocarcinoma has drastically increased the last three to five decades becoming the predominant subtype [6, 7], Figure 3B-C. In the last decades the global incidence of esophageal squamous cell carcinoma has started to decline, although for women there has been an increase in some developed countries [8].

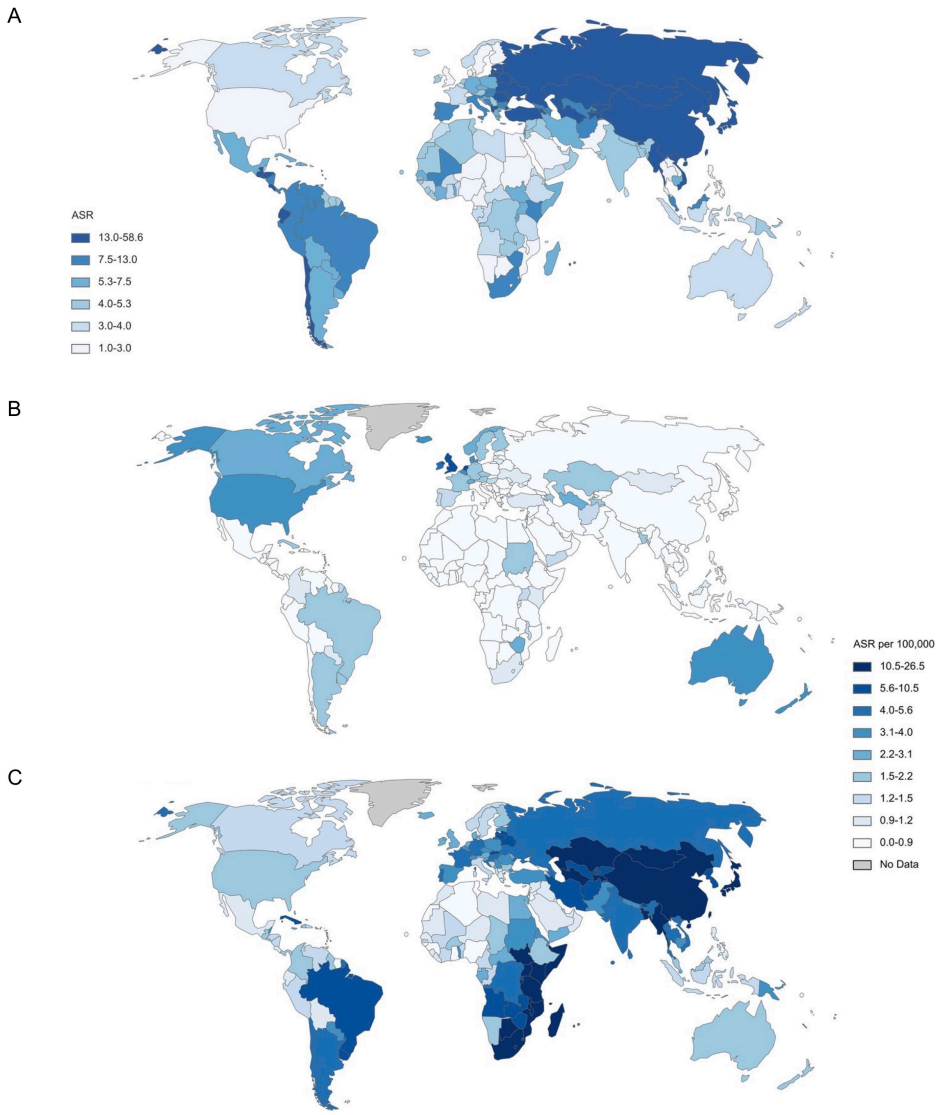

Figure 3.
Age-standardized incidence rate (ASR) per 100,000 men in 2012 for (A) non-cardia gastric cancer, (B) esophageal adenocarcinoma and (C) esophageal squamous cell carcinoma. The incidence in women is lower than in men but with a similar geographical distribution. Reproduced from (3A) Colquhoun et al. [4] and (3B-C) Arnold et al. [6] with permission from BMJ Publishing Group Ltd.

In Sweden, with a population of a little over 10 million, the average annual incidence 2013-2017 of gastric and esophageal cancer was approximately 1200 new cases; for gastric cancer (adenocarcinoma): 264 men (ASR 2.3/100,000) and 196 women (ASR 1.6/100,000); for esophageal (including GE junction) adenocarcinoma: 418 men (ASR

4.0/100,000) and 107 women (ASR 0.9/100,000); for esophageal squamous cell carcinoma: 121 men (ASR 1.1/100,000) and 78 women (ASR 0.6/100,000) [9].

Etiology

A common denominator for esophageal and gastric cancer is the relationship with increasing age, male gender and smoking.

For gastric cancer there are several dietary risk factors such as intake of salty (historically used for preservation) food [10], red and processed meat [11] and low intake of fruits and vegetables [12]. Heavy, but not moderate, alcohol drinking is also a risk factor [13]. The gram negative bacteria *Helicobacter pylori* discovered 1982 [14], colonizing the human stomach, is associated with chronic gastritis and gastric cancer. Due to the overall prevalence of *H. pylori* in 51% of the population in developing countries and 35% in developed countries [15] it constitutes a major risk factor. It is estimated that almost 90% of gastric cancer cases worldwide are attributable to *H. pylori* [16]. Another pathogen, the Epstein-Barr virus (EBV), is a rare but known risk factor for gastric cancer [17]. Low socioeconomic status measured as level of education and household income is associated with a higher incidence of gastric cancer, independent of *H. pylori* infection [18]. About 10% of gastric cancers have familial clustering but only 1-3% is thought to have a hereditary genetic cause [19]. Individuals carrying a germline mutation of the *CDH1* gene, encoding the cell-adhesion protein E-cadherin, have a very high lifetime risk (up to 70%) of hereditary diffuse gastric cancer [20] why prophylactic gastrectomy is often considered [21]. Other genetic syndromes with increased gastric cancer risk are Peutz-Jeghers, juvenile polyposis, Lynch, Li-Fraumeni and variants of familial adenomatous polyposis [19, 22].

Two major risk factors for esophageal adenocarcinoma are gastroesophageal reflux disease [23] and obesity [24]. Alcohol intake does not seem to be a risk factor [25] and *H. pylori* infection is suggested to be protective [26] as is intake of fruits and vegetables [27]. Individuals with low socioeconomic status are at higher risk of esophageal adenocarcinoma [28]. Although not associated with any of the major hereditary syndromes, esophageal adenocarcinoma and the precursor Barrett's esophagus can have a familial clustering [29] and genetic predisposition [30].

For esophageal squamous cell carcinoma smoking is a major risk factor, particularly in developed countries [31]. Other risk factors are alcohol and red and processed meat whereas fruits and low body mass index appear to be protective [32]. Low socioeconomic status is associated with an increased incidence [33].

Classification and pathogenesis

In 1965 Pekka Laurén proposed a still widely used histological classification of gastric adenocarcinoma with two subtypes: intestinal and diffuse type, respectively [34]. The intestinal type is characterized by cohesive and differentiated cells, *H. pylori* infection, male predominance, declining incidence, distal gastric location and hematogenous dissemination (particularly to liver and lungs), whereas the diffuse type is characterized by discohesive and poorly differentiated cells (sometimes abundant in mucin), female predominance, rising incidence, younger age at diagnosis, peritoneal spread, lower chemosensitivity and a poor prognosis [35–38]. Of note, a few percent of gastric malignancies are not adenocarcinomas, e.g. lymphomas, sarcomas and neuroendocrine cancers.

Histological subclassification of esophageal cancer is usully restricted to a separation between squamous cell carcinoma and adenocarcinoma, although the Laurén classification is sometimes applied on the latter [39]. The vast majority of the adenocarcinomas are located in the distal part of the esophagus including the GE junction whereas the squamous cell carcinomas can be located anywhere in the esophagus, Figure 4.

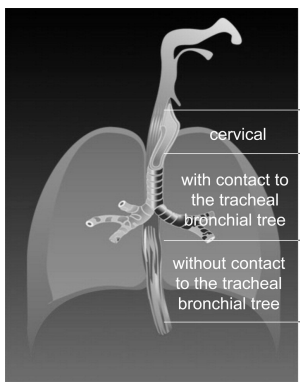

Figure 4.
Differences in tumor location of adenocarcinoma (Adeno-Ca) and squamous-cell carcinoma (SCC) of the esophagus in a large German surgical cohort. Reprinted from Siewert et al. (Siewert 2007)) with permission from Elsevier.

Diffuse type gastric cancer have no obvious precursor lesion and the cancer cells develop de novo from the normal epithelium. Intestinal type gastric cancer and esophageal adenocarcinoma, on the other hand, evolve through a multistep morphological process where a chronic inflammation (e.g. *H. pylori* infection in the stomach) or irritant (acid or bile reflux in the distal esophagus), in combination with genetic, dietary and environmental factors, result in replacement of the normal epithelium with an intestinal metaplasia (called Barrett's in the esophagus) and then, via dysplasia and intramucosal

cancer, eventually an invasive adenocarcinoma develops [40]. Esophageal squamous cell carcinoma develops through increasing grade of dysplasia [41].

In recent years a deepened understanding of the genetic and epigenetic mechanisms of esophageal and gastric cancer has evolved and new molecular classifications have been proposed, e.g. The Cancer Genome Atlas (TCGA). The TCGA classification, based on different molecular platforms (whole-exome sequencing, profiling of somatic copy-number alterations and DNA methylation, sequencing of mRNA and microRNA and proteomics), identifies five different subtypes: Epstein-Barr virus (EBV), microsatellite instability (MSI), genomically stable (GS), chromosomal instability (CIN) and esophageal squamous cell carcinoma (ESCC) [42, 43], Figure 5.

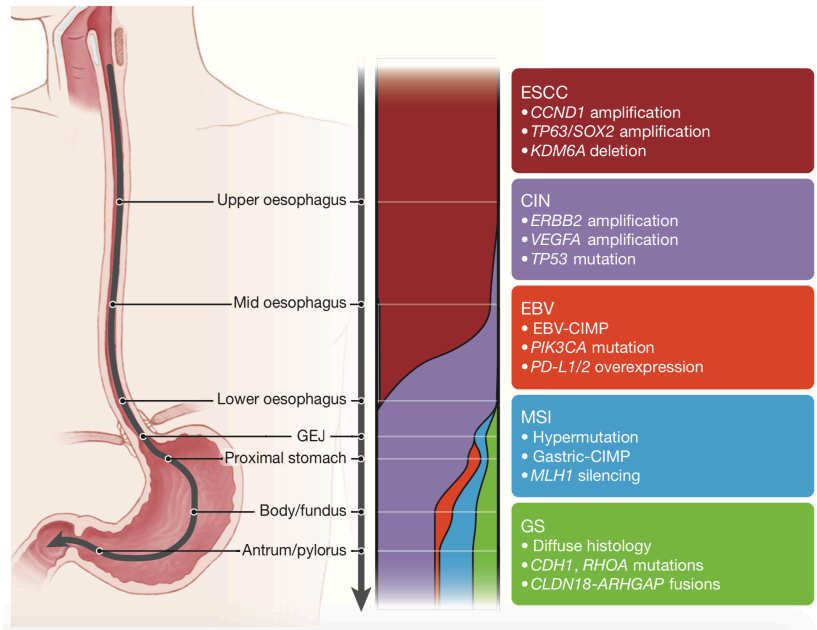

Figure 5.
The TCGA molecular subtypes and key features of gastric and esophageal cancer. Reprinted from Nature[43] with permission from Springer under the terms of http://creativecommons.org/licenses/by/4.0/.

Clinical presentation, diagnosis and work-up

Dysphagia (i.e. difficulty in swallowing) is the most common presenting symptom in patients with esophageal cancer. Less common initial symptoms are reflux, dyspepsia, anorexia, pain, nausea, vomiting, dyspnea, bleeding and anemia [44–46]. For patients with gastric cancer there are no typical early symptoms or signs, but eventually any of the symptoms described for esophageal cancer can occur as well as early satiety [47, 48].

Upper endoscopy (esophagogastroduodenoscopy) with biopsies is the gold standard for initial diagnosis of esophageal and gastric cancer. Staging is done using computed tomography (CT) of the chest, abdomen and pelvis, with or without ^{18}F-fluorodeoxyglucose (FDG) positron emission tomography (PET). Endoscopic ultrasound is sometimes used to further complemement the staging of the primary tumor and nodal status. Staging laparoscopy, particularly in gastric cancer, can be useful to rule out peritoneal carcinomatosis [49, 50].

For patients that are candidates for major surgery it is often recommended to perform respiratory and cardiac function tests, e.g. spirometry and exercise-electrocardiography, to assure that they are fit for surgery [51].

Optimized nutrition and psychosocial support is of utmost importance regardless of the treatment intent [52, 53]. For every new patient it is recommended that the diagnosis, staging and treatment options are discussed in a multidisciplinary team meeting to improve staging accuracy and treatment recommendations [54].

Staging

Esophageal and gastric cancer are staged using the UICC/AJCC TNM classification system where the T category refers to the invasive depth of the primary tumor, the N category refers to lymph node metastases, and the M category refers to distant metastases, Figure 6. These categories can then be combined into prognostic stage groups (not shown in here). The current TNM classification is the 8th edition but in this thesis the 7th edition [55], Table 1, was used in all papers.

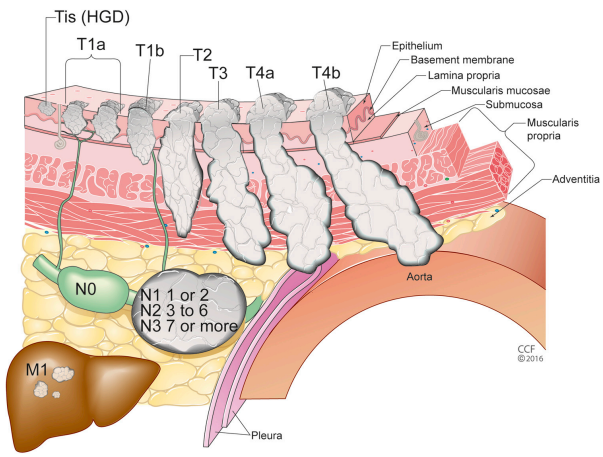

Figure 6.
Illustration of the T, N and M categories in esophageal cancer. In gastric cancer the categories are almost the same but the stomach is surrounded by a serosa instead of an adventitia. Reprinted from Rice et al. [56] with permission from Elsevier.

Table 1.
TNM classification, 7th edition.

Esophageal (and GE junction) cancer	
TX	Primary tumor cannot be assessed
T0	No evidence of primary tumor
Tis	Carcinoma in situ/high-grade dysplasia
T1	Tumor invades lamina propria, muscularis mucosae, or submucosa
T1a	Tumor invades lamina propria or muscularis mucosae
T1b	Tumor invades submucosa
T2	Tumor invades muscularis propria
T3	Tumor invades adventitia
T4	Tumor invades adjacent structures
T4a	Resectable tumor invading pleura, pericardium or diaphragm
T4b	Unresectable tumor invading other adjacent structures such as aorta, vertebral body, trachea, etc
NX	Regional lymph nodes cannot be assessed
N0	No regional lymph node metastasis
N1	Metastases in 1-2 regional lymph nodes
N2	Metastases in 3-6 regional lymph nodes
N3	Metastases in ≥7 regional lymph nodes
M0	No distant metastasis
M1	Distant metastasis

Gastric cancer	
TX	Primary tumor cannot be assessed
T0	No evidence of primary tumor
Tis	Carcinoma in situ/high-grade dysplasia
T1	Tumor invades lamina propria, muscularis mucosae, or submucosa
T1a	Tumor invades lamina propria or muscularis mucosae
T1b	Tumor invades submucosa
T2	Tumor invades muscularis propria
T3	Tumor penetrates subserosal connective tissue
T4	Tumor invades serosa (visceral peritoneum) or adjacent structures
T4a	Tumor invades serosa (visceral peritoneum)
T4b	Tumor invades serosa adjacent structures such as spleen, transverse colon, liver, diaphragm, pancreas, abdominal wall, adrenal gland, kidney, small intestine, peritoneum
NX	Regional lymph nodes cannot be assessed
N0	No regional lymph node metastasis
N1	Metastases in 1-2 regional lymph nodes
N2	Metastases in 3-6 regional lymph nodes
N3	Metastases in ≥7 regional lymph nodes
M0	No distant metastasis
M1	Distant metastasis

Prognostic factors

As mentioned above, the TNM classification can be used for prognostication, particularly the current 8th edition which has separate classifications for the pretreatment clinical cTNM, the pathological pTNM and the pathological after neoadjuvant treatment ypTNM.

In a recent meta-analysis [57] of randomized trials on curative treatment, 16 prognostic factors were identified for esophageal cancer and 23 for gastric cancer, e.g. age, comorbidity, TNM categories, radicality, differentiation grade, MSI, nutritional status, body weight, hospital resection volume, etc. In the palliative setting of esophageal and gastric cancer, a meta-analysis [58] from the same group identified 17 prognostic factors in the first-line treatment setting, e.g. performance status, locally advanced vs. metastatic, recurrent vs. unresectable at diagnosis, intestinal type vs. diffuse type, number of metastatic sites, etc.

In addition, there are numerous proposed prognostic biomarkers but none used in routine clinical practice.

Treatment of localized disease

Not only the diagnosis and staging guides the choice of treatment but also the patient's general condition, comorbidites and personal preferences.

Early disease

Superficial lesions, i.e. high grade dysplasia (carcinoma in situ) or T1a cancer are best treated with endoscopic mucosal resection (EMR) or endoscopic submucosal dissection (ESD) and/or local ablation [59].

For early esophageal cancer (T1b-T2) or gastric cancer (T1b), without suspicion of lymph node metastases (N0), surgical resection merely is usually the recommended treatment for fit patients [50, 60, 61]. The surgical approach has historically been open surgery but thoracoscopic and laparoscopic techniques are increasingly being used. For esophageal cancer an esophagectomy is the gold standard whereas for gastric cancer the choice of total or partial gastrectomy depends on the tumor location and the histological subtype (diffuse type requires larger margins). The extent of lymph node dissection has for long been a matter of debate in both esophageal (two-field vs. three-field) and gastric (D1 vs. D2) cancer, balancing radicality vs. morbidity, with a preference for more extended resections in Asia [62, 63].

Locally advanced gastric cancer

The American INT 0116 trial [64], published in 2001, on gastric (81%) and GE junction adenocarcinoma cancer demonstrated a superior overall survival with adjuvant chemoradiotherapy (fluorouracil + 45 Gy) compared to surgery alone and this concept was widely adopted in the United States but not in Europe where the trial was criticized for the limited lymph node dissections performed.

The pivotal UK MAGIC trial [65], published in 2006, on resectable gastroesophageal adenocarcinoma (74% gastric cancer) compared surgery alone with perioperative (i.e. neoadjuvant + adjuvant) chemotherapy using epirubicin, cisplatin and fluorouracil (ECF regimen), three cycles before and three cycles after surgery. The perioperative approach yielded a superior 5-year overall survival compared to surgery alone (36% vs. 23%), HR 0.75 (95% CI, 0.60-0.93) and this treatment was rapidly introduced in most European countries, although in Sweden it was swiftly modified to the more convenient EOX regimen (epirubicin, oxaliplatin and capecitabine) based on the REAL2 trial [66] which in the metastatic setting demonstrated a longer survival with EOX compared to ECF. The French study FFCD 9703 [67], published in 2011, on resectable gastroesophageal adenocarcinoma (25% gastric cancer) had a design similar to the MAGIC trial, but without epirubicin, and the 5-year overall survival was 38% in the perioperative arm and 24% in the surgery only arm, HR 0.69 (95% CI 0.50–0.95). In later years these perioperative chemotherapy trials have been criticized for poor quality of surgery and methodological shortcomings [68, 69]. In 2017 the results from the German FLOT4 trial [70] was presented, establishing FLOT (fluorouracil, calcium folinate, oxaliplatin and docetaxel) as the new standard perioperative chemotherapy regimen in gastric and GE junction adenocarcinoma with an estimated 5-year overall survival of 45% for FLOT and 36% for ECF/ECX, HR 0.77 (95% CI, 0.63-0.94).

The role of adjuvant chemoradiotherapy in the era of perioperative chemotherapy in gastric and GE junction adenocarcinoma was addressed in the Dutch CRITICS trial [71] comparing neoadjuvant chemotherapy and adjuvant chemoradiotherapy with perioperative chemotherapy but with no differences in survival. An inverse strategy, with neoadjuvant chemoradiotherapy and adjuvant chemotherapy vs. perioperative chemotherapy is currently being investigated in the Australian TOP GEAR trial [72].

In Asia (particularly in Japan and South Korea) the standard approach in gastric cancer is surgery up-front followed by adjuvant chemotherapy. This is based on the ACTS-GC trial [73] with adjuvant S-1 (tegafur/gimeracil/oteracil) and the CLASSIC trial [74] with adjuvant capecitabin and oxaliplatin (CAPOX), both demonstrating improved survival rates compared to surgery alone. In Western populations there are no large studies supporting adjuvant chemotherapy alone although there is some evidence from a meta-analysis on smaller trials [75]. Recently, at the ESMO meeting 2019, the Asian RESOLVE trial [76] on stage T4 locally advanced gastric cancer demonstrated a

superior disease-free survival with perioperative SOX (S-1 and oxaliplatin) compared to adjuvant CAPOX.

Locally advanced esophageal cancer

The Dutch CROSS trial [77, 78], published in 2012, established neoadjuvant chemoradiotherapy (paclitaxel + carboplatin + 41.4 Gy) as a new standard treatment with a 5-year overall survival of 47% vs. 33% for the neoadjuvant approach compared to surgery only. The majority of tumors were adenocarcinomas and located in the distal esophagus or GE junction but the survival benefit of neoadjuvant chemoradiotherapy was much larger for squamous cell carcinomas, HR 0.48 (95% CI 0.28-0.83), than for adenocarcinomas, HR 0.73 (95% CI 0.55-0.98).

Since patients with distal esophageal (including the GE junction) adenocarcinomas were included in the perioperative chemotherapy trials (MAGIC, FFCD 9703, FLOT4), mentioned in the section above on gastric cancer, this is also a standard treatment option for patiens with resectable esophageal adenocarcinoma. To date it is unknown whether neoadjuvant chemoradiotherapy ad modum CROSS or perioperative chemotherapy is the best option but there are two ongoing phase III trials, ICORG 10-14/Neo-AEGIS [79] and ESOPEC [80], investigating this issue. Smaller randomized trials comparing older variants of neoadjuvant chemoradiotherapy with neoadjuvant chemotherapy have not revealed any significant survival advantages for either strategy [81–83].

Another treatment alternative in locally advanced esophageal cancer, particularly for squamous cell carcinoma, is definitive chemoradiotherapy. The American study RTOG 8501 [84] randomizing between chemoradiotherapy (fluorouracil + cisplatin + 50 Gy) and radiotherapy alone (64 Gy) demonstrated a 5-year overall survival of 26% vs. 0% favoring the combined treatment. Escalation of the radiotherapy dose in definitive chemoradiotherapy was investigated in INT 0123/RTOG 9405 [85] but with no survival benefit for 64.8 Gy vs. 50.4 Gy. The French study PRODIGE 5/ACCORD 17 [86] with definitive chemoradiotherapy to 50 Gy comparing standard fluorouracil + cisplatin with the FOLFOX4 (fluorouracil + calcium folinate + oxaliplatin) regimen showed no survival differences but fewer toxic deaths in the FOLFOX4 group. In all these trials approximately 85% of the tumors were squamous cell carcinomas. Definitive chemoradiotherapy for adenocarcinomas has been much less investigated and cannot be routinely recommended. Small trials [87, 88] comparing definitive chemoradiotherapy with neoadjuvant chemoradiotherapy followed by surgery have not shown any statistical survival differences but a lower rate of local control with the former approach, thus the trimodal approach is usually the preferred option for fit patients. However, a large retrospective study [89] on the role of salvage surgery after local failure of definitive chemoradiotherapy demonstrated encouraging long-term

survival rates and the forthcoming NEEDS trial on esophageal squamous cell carcinoma will compare neoadjuvant chemoradiotherapy + surgery with definitive chemoradiotherapy followed by vigilant surveillance and salvage surgery as needed.

Summary of current European treatment recommendations for locally advanced gastric and esophageal cancer in fit patients

The standard approach for gastric cancer is perioperative chemotherapy where FLOT is the new reference regimen. For esophageal adenocarcinoma either perioperative FLOT or neoadjuvant chemoradiotherapy ad modum CROSS is recommended and for squamous cell carcinoma chemoradiotherapy, either as neoadjuvant or as definitive treatment, are the standard options.

Chemotherapy issues in the perioperative setting

Prediction of benefit

Based on the 5-year overall survival rates in the MAGIC, FFCD 9703 and FLOT4 trials mentioned above, merely about 15-20% of the patients may actually benefit from the addition of perioperative chemotherapy to surgery. Thus, the majority of the patients will receive a toxic treatment that will not help them. Unfortunately, there are hitherto no established tools to identify which patient who is likely to benefit from perioperative chemotherapy, although several candidate biomarkers have been proposed.

MSI or dMMR (deficient mismatch repair) tumors have in several studies been suggested to be insensitive to chemotherapy. In post hoc analyses of the MAGIC trial [90] and the adjuvant CLASSIC trial [91] no benefit of fluoropyrimidine and platinum based chemotherapy could be shown for the small proportion (~ 7%) of patients with MSI tumors. However, a large retrospective German study on neoadjuvant chemotherapy could not confirm MSI to be predictive [92]. Other proposed, potentially predictive biomarkers are DNA methylation [93] or gene expression signatures [94], polymorphism of drug metabolism genes [95], expression of DNA repair genes [96] or signatures involving tumor immune cell infiltration [97].

Relative dose intensity

Delivering perioperative triplet chemotherapy, such as ECF/ECX/EOX or FLOT, can take a heavy toll on the patients due to side effects, comorbidities and poor (especially

after major surgery) performance status. In daily clinical practice dose reductions, treatment delays and even premature discontinuation are common [69, 98] but little is known how these modifications affect outcome.

A common method to assess chemotherapy delivery is the Hryniuk [99] model of relative dose intensity (RDI), i.e. the ratio of actual to planned dose intensity where dose intensity is the cumulative dose divided by the total treatment duration.

In resected gastric cancer there are two retrospective studies [100, 101] on RDI of adjuvant chemotherapy (S-1), both demonstrating an association between RDI and survival. We are not aware of any studies on the impact of RDI of perioperative or neoadjuvant chemotherapy on outcome, neither in gastric nor esophageal cancer. It is also unknown whether it should be recommended to reduce the doses or to delay the treatment in case of poor tolerance to chemotherapy.

Palliative treatment

For patients with metastatic disease or patients with localized disease but who are unfit for curative treatment, palliative oncological treatment should be considered along with best supportive care.

Systemic treatment of esophageal and gastric adenocarcinoma

In many of the phase III chemotherapy trials both gastric and GE junction adenocarcinoma patients were included together and, since the majority of the esophageal adenocarcinomas are located in the GE junction, gastric and esophageal adenocarcinomas are often considered as a singel disease entity in this context.

Early trials comparing chemotherapy with best supportive care alone have demonstrated a prolonged median overall survival of approximately six months with chemotherapy [102], although in Western populations the median overall survival is usullay less than a year. The chemotherapy backbone in first-line treatment is a fluoropyrimidine (fluorouracil, capecitabine or S-1) combined with a platinum compound (cisplatin or oxaliplatin). Several trials have shown that fluorouracil can be replaced with an oral fluoropyrimidine (capecitabine or S-1) and that cisplatin can be replaced with oxaliplatin, maintaining at least similar efficacy [66, 103–106]. A fluoropyrimidine combined with irinotecan is another option in the first-line setting [107, 108]. For patients with HER2 (Human epidermal growth factor receptor 2) positive (7-34% of EGAC) gastric or GE junction adenocarcinoma the ToGA trial [109] showed that the addition of trastuzumab to a fluoropyrimidine and cisplatin increased median overall survival with several months, especially for those with strong

HER2 positivity. The addition of a third cytotoxic drug, e.g. docetaxel, in the first-line setting can increase efficacy but at the risk of substantial toxicity [110]. Recently, the UK GO2 trial [111] on palliative capecitabine + oxaliplatin in frail and/or elderly patients comparing full dose, 80% dose and 60% dose, showed that the lowest dose yielded better quality of life without compromising survival.

Patients that maintain a good performance status after failure of first-line treatment might benefit from second-line treatment. Monotherapy with docetaxel, paclitaxel or irinotecan prolongs median overall survival with one and a half month compared to best supportive care only [112–114]. The RAINBOW study [115] demonstrated that addition of ramucirumab (anti-VEGFR2) to paclitaxel increased median overall survival with 2.2 months and this is now standard of care in many countries. Recently, the TAGS study [116] showed that, for patients failing at least two treatment lines, trifluridine/tipiracil compared to placebo prolonged median overall survival with 2.1 months and this treatment should be considered for those very few patients that are still in good condition.

Systemic treatment of esophageal squamous cell carcinoma

The evidence for benefit from palliative chemotherapy in patients with esophageal squamous cell carcinoma is weaker than for adenocarcinoma. A combination of fluoropyrimidine and platinum is considered standard of care for fit patients, although old and small (and underpowered) trials have not shown any survival benefit with chemotherapy compared to observation [117, 118].

Immunotherapy in esophageal and gastric cancer

In Europe there are currently no approved immunotherapies in gastric or esophageal cancer, however, based on recently reported and ongoing phase III trials in Western populations they might be just around the corner for certain tumor subgroups.

In the first-line setting of PD-L1 positive gastric or GE junction adenocarcinoma the KEYNOTE-062 trial [119] showed no survival differences between pembrolizumab vs. chemotherapy nor between pembrolizumab + chemotherapy vs. chemotherapy. However, in the subgroup of patients (36%) with high expression of PD-L1 (combined prognostic score (CPS) $\geq$ 10), the median overall survival was 17.4 vs. 10.8 months and the 2-year overall survival was 39% vs. 22% for pembrolizumab vs. chemotherapy.

In the KEYNOTE-181 trial [120] on second-line pembrolizumab vs. chemotherapy in esophageal cancer (adenocarcinoma or squamous cell carcinoma) there was no survival advantage with pembrolizumab in the whole study population but in the subgroup of patients (35%) with PD-L1 CPS $\geq$ 10 there was an advantage for pembrolizumab vs. chemotherapy with a median overall survival of 9.3 vs. 6.7 months.

Treatment of dysphagia

Dysphagia is the predominant symptom in most patients with incurable esophageal cancer, severely affecting quality of life, nutritional status and body weight [121, 122]. Thera are several treatment options to alleviate dysphagia, e.g. insertion of a self-expanding metal stent (SEMS), intraluminal brachytherapy, external beam radiotherapy, chemoradiotherapy, chemotherapy and various endoscopic local ablative therapies with laser, cryotherapy, photodynamic therapy, argon plasma coagulation or ethanol injection [123, 124]. The most commonly utilized local interventions are SEMS or radiotherapy (external or, if available, intraluminal). With SEMS placement dysphagia can be relieved within a week but the improvement typically only lasts for a few months due to tumor ingrowth or SEMS displacement, thus it is usually the preferred option for patients with severe dysphagia or a short life expectancy. Radiotherapy on the other hand has a delayed (~ 6 weeks) onset of dysphagia relief but with a more sustained duration of improvement compared to SEMS [124–126], Figure 7-8. In all prospective trials on palliative external beam radiotherapy in esophageal cancer the total dose has been 30 Gy or higher, delivered in ten or more fractions [127–135]. A small trial [130] has shown that combining SEMS insertion and external beam radiotherapy is safe and beneficial but results from the randomized phase III ROCS trial [136] are yet to be reported.

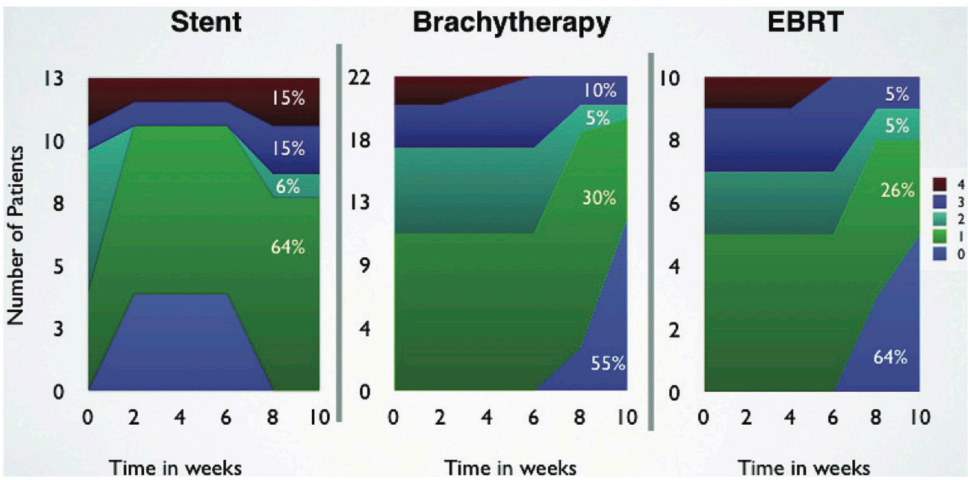

Figure 7.
Dysphagia score from 0 (no dysphagia) to 4 (complete dysphagia) over time in a small retrospective study [124] on patients with esophageal cancer treated with SEMS, brachytherapy or external beam radiotherapy (EBRT). Reprinted with permission from Multimed.

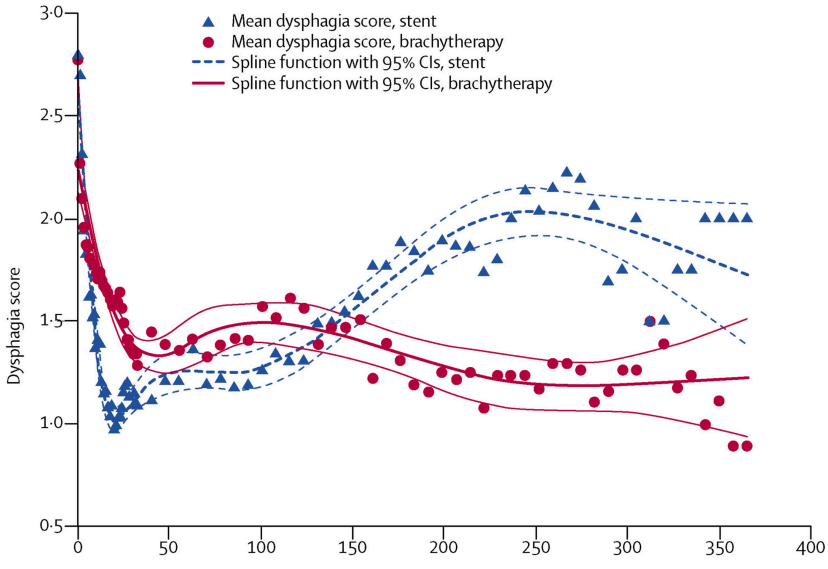

Figure 8.
Mean dysphagia score over time in a randomized trial [126] comparing SEMS with single-dose brachytherapy (12 Gy). Reprinted with permission from Elsevier.

Podocalyxin-like protein 1

Podocalyxin like protein 1 (PODXL), a member of the CD34 family, is a transmembrane cell surface glycoprotein, Figure 9, involved in regulation of cell adhesion and morphology. PODXL is encoded by the *PODXL* gene on chromosome 7q32-q33. Proposed negative regulators of *PODXL* gene expression are *TP53* [137], Krüppel-like factor 4 [138] and methylation of the *PODXL* promoter [139], whereas Wilms tumor suppressor-1 (*WT1*), despite its name, is a positive regulator [137]. In addition, RNA misediting of the *PODXL* transcript can cause functional alteration of PODXL [140].

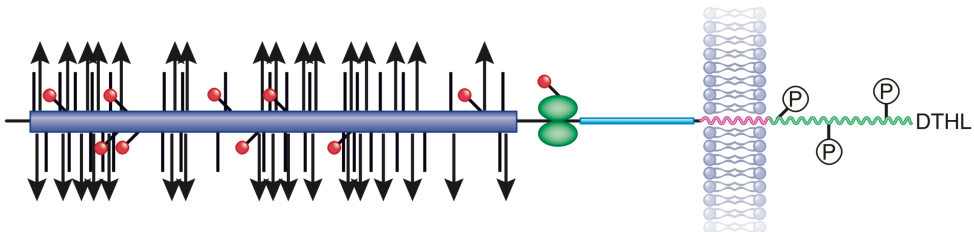

Figure 9.
PODXL is composed of a extracellular mucin domain rich in *O*-linked glycans (vertical lines), sialic acid (triangles) and *N*-linked glycans (lines with red circles). Further there is a globular domain (green), a juxtamembrane stalk (blue), a transmembrane portion (pink) and a cytoplasmic tail (green) with phosphorylation sites (P). DTHL is an aminoacid sequence. Reprinted from Nielsen et al. [141] with permission from American Society of Nephrology.

PODXL was first described in 1984 as a 140 kilodalton protein in the glycocalyx of kidney podocytes [142] in which it is involved in regulation of the glomerular filtration [143]. Eventually it was found to be expressed in vascular endothelial cells [144], hematopoetic progenitor cells [145] and in developing neurons [146]. PODXL has mainly been considered as an anti-adhesive protein but can also be pro-adhesive, e.g. in the adhesion of leucocytes to high endothelial venules (promoting recruitment) in lymph nodes [147] or cell binding to platelets [148].

The first description of PODXL in malignant cells was in non-seminomatous testicular cancer [149]. Since then, overexpression of PODXL has been found in a wide range of malignancies and associated with an aggressive phenotype and poor prognosis e. g. in breast cancer [150], colorectal cancer [151–153], pancreatic and periampullary cancer

[154–156], bladder cancer [157], glioblastoma multiforme [158] and oral squamous cell carcinoma [159].

When we initated our studies on PODXL there were no reports in gastric or esophageal cancer, but prior to our first article (Paper I) Laitinen et al. [160] reported an association between PODXL expression and poor prognosis in gastric cancer. Except for our reports herein (Paper I and II) there are still no other publications on PODXL in esophageal adenocarcinoma.

The functional mechanism of PODXL in malignancy has began to be revealed and PODXL has been shown to enhance proliferation, invasion, migration and the metastatic potential of tumor cells, in vitro and in vivo, presumably by epithelial-mesenchymal transition (EMT) [138, 161–165]. EMT is the process where epithelial cells gradually attain a mesenchymal-like phenotype enabling loss of cell-cell adhesion, invasion through the basement membrane and extracellular matrix into the tissue and eventually, for cancer cells, dissemination via blood or lymphatic vessels [166], Figure 10.

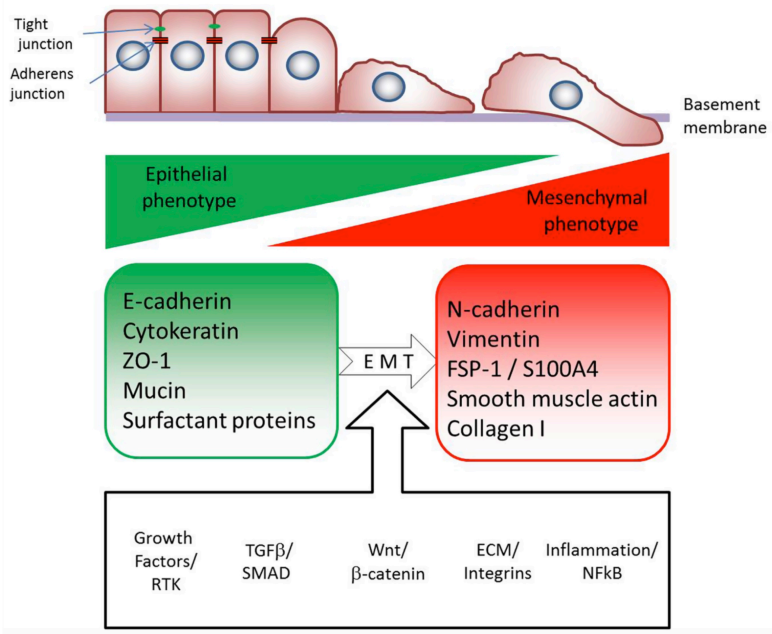

Figure 10.
Features of epithelial-mesenchymal transition. Reprinted from Bartis et al. [167] with permission from BMJ Publishing Group Ltd.

Transforming growth factor-β, a major inducer of EMT, has in lung cancer cells been shown to exert its effect via PODXL [161]. In the same study loss of E-cadherin and increase in vimentin, typically associated with EMT, was not as obvious when the

PODXL gene was silenced, thus indirectly supporting the role of PODXL in EMT. In breast and prostate cancer cells, PODXL has been shown to interact with the actin-binding protein ezrin, activating the MAPK/ERK and PI3K/AKT pathways, and increasing the expression of matrix metalloproteases, thereby enhancing tumor cell migration and invasion [168]. In gastric cancer cells, PODXL has been shown to activate PI3K/AKT, NF-κB and MAPK/ERK pathways, thus potentially enhancing cell proliferation and migration. Overexpression of PODXL also increased the expression of the anti-apoptotic protein Bcl-2 and matrix metalloproteases, whereas the levels of pro-apoptotic Caspase and Bax were decreased [169]. Other suggested mechanisms of PODXL in tumorigenesis are immune evasion [170] or stabilization of glucose transporters [171]. Furthermore, in various cell lines from colon cancer, osteosarcoma, oral tongue squamous cell carcinoma and astrocytoma, PODXL expression has been linked to insensitivity to chemotherapy, e.g. fluorouracil, irinotecan, cisplatin and temozolomide [163, 172–174].

Aims of the thesis

The overall aim was to improve the oncological treatment strategies in esophageal and gastric adenocarcinoma.

Specific aims:

- To investigate the potential prognostic impact of PODXL expression in resected esophageal and gastric adenocarcinoma (Paper I)
- To assess if PODXL expression could be a predictive biomarker to identify patients who will benefit from neoadjuvant ± adjuvant chemotherapy in esophageal and gastric adenocarcinoma (Paper II)
- To assess how dose reductions and treatment delays affect histopathologic response in esophageal and gastric adenocarcinoma treated with neoadjuvant EOX (Paper III)
- To investigate if sequential short-course radiotherapy with 20 Gy in five fractions, followed by chemotherapy (FOLFOX), is a promising treatment strategy to achieve long-term relief of dysphagia in patients with incurable esophageal adenocarcinoma (Paper IV)

Material and methods

Patients

Cohort 1

To assess the potential prognostic role of PODXL (Paper I) we used a cohort of 174 consecutive patients with esophageal or gastric adenocarcinoma treated with surgical resection up-front (no neoadjuvant treatment) at the University Hospitals of Lund and Malmö between January 1, 2006 and December 31, 2010. Only a minority (7%) of the patients received adjuvant treatment. Data on survival status and recurrence were updated until December 31, 2014 (Paper I) and until March 1, 2016 (Paper II).

Cohort 2

To assess the potential predictive role of PODXL (Paper II) we assembled a new cohort of 148 consecutive patients with resectable esophageal or gastric adenocarcinoma who started neoadjuvant chemotherapy at the Skåne University Hospital (a merger of the University Hospitals in Lund and Malmö) between January 1, 2008 and December 31, 2014. Follow-up was done until December 31, 2017. The resected patients from this cohort were then merged with the patients from cohort 1 into a pooled cohort.

To assess the impact of dose reduction and treatment delay of neoadjuvant chemotherapy (Paper III) we focused on 63 patients from cohort 2 who were treated with neoadjuvant EOX followed by surgical resection and for whom we hade detailed data of chemotherapy delivery.

Cohort 3

In the phase II PALAESTRA trial (Paper IV) 29 patients with treatment-naîve esophageal or GE junction adenocarcinoma, not eligible for curative treatment, were enrolled at the Skåne University Hospital from October 3, 2014 to May 9, 2018. Key eligibility criteria were age 18 years or older, WHO performance status 0-2, dysphagia score 1 or worse, no SEMS in situ, life expectancy longer than three months and signed written informed consent. Data cutoff date was May 17, 2019.

In all cohorts classification of tumor stage was done according to the 7th edition of the UICC/AJCC TNM classification, thus tumors in the GE junction Siewert type I–III were classified as esophageal cancers. Residual tumor status (Cohort 1 and 2), i.e. the radicality, was denoted as: R0 = no residual tumor (free resection margins according to the pathology report), R1 = possible microscopic residual tumor (narrow or compromised resection margins according to the pathology report), R2 = macroscopic residual tumor (according to the operative report).

Assessment of PODXL expression

From cohort 1 and 2 archival blocks with formalin-fixed paraffin embedded tissue were obtained as well as pre-neoadjuvant diagnostic biopsies from cohort 2. Except for the biopsies that were analyzed in full-face sections, we used tissue microarrays (TMA), Figure 11, where duplicate cores (1 mm in diameter) from donor blocks, with tissue from primary tumors, lymph node metastases, intestinal metaplasia and benign epithelium, were collected and arranged in recipient blocks. For subsequent immunohistochemistry, 3 µm sections from the biopsies and 4 µm sections from the TMAs were prepared and stained with the rabbit polyclonal anti-PODXL antibody HPA002110 (Atlas Antibodies AB, Stockholm, Sweden).

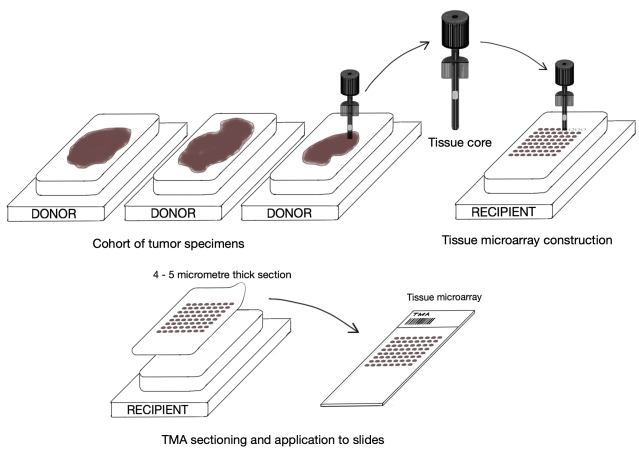

Figure 11.
Construction of a tissue microarray where cores from donor blocks are arranged in a recipient block and then further processed. Reprinted courtesy of Dr Gustav Andersson.

PODXL staining was scored by two observers and for duplicate cores the highest staining score was used. The scores were trichotomized and dichotomized as described in Table 2.

Table 2.
Classification of PODXL expression.

Staining	Score	Trichotomized	Dichotomized
Negative	0	Negative	Negative
Weak cytoplasmic positivity in any proportion of cells	1	Low	Positive
Moderate cytoplasmic positivity in any proportion of cells	2		
Distinct membranous positivity in ≤ 50% of cells	3	High	
Distinct membranous positivity in > 50% of cells	4		

Histopathologic response

In paper II and III the extent of residual cancer cells in the primary tumor site, after neoadjuvant chemotherapy and resection, was histologically evaluated using the four-tiered tumor regression grading system described by Chirieac [175], i.e. 0%, 1–10%, 11–50% or > 50% residual cancer cells, Figure 12.

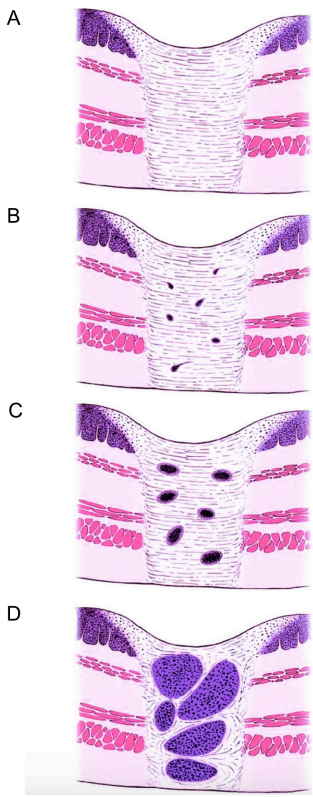

Figure 12.
Illustration of histopathologic response in the primary tumor site with (A) no residual cancer cells, (B) 1-10% residual cancer cells, (C) 11-50% residual cancer cells and (D) >50% residual cancer cells. Reprinted from Chirieac et al. [175] with permission from Wiley.

Relative dose intensity, dose index and time index of neoadjuvant EOX

In paper III, the individual factors of RDI, i.e. dose index (DI) and time index (TI), were calculated for each patient as described by Nakayama et al. [176]. EOX DI, TI and RDI are composite measures of mean values of the three individual drugs in the EOX regimen.

$$\boldsymbol{Dose\ intensity} = \frac{\boldsymbol{Cumulative\ dose}\ (mg/m^2)}{\boldsymbol{Total\ duration}\ (week)}$$

$$\boldsymbol{Relative\ dose\ intensity\ (RDI)} = \frac{\boldsymbol{Actual\ dose\ intensity}}{\boldsymbol{Planned\ dose\ intensity}}$$

$$= \frac{\frac{Actual\ cumulative\ dose}{Actual\ total\ duration}}{\frac{Planned\ cumulative\ dose}{Planned\ total\ duration^*}} = \frac{Actual\ cumulative\ dose \times Planned\ total\ duration^*}{Planned\ cumulative\ dose \times Actual\ total\ duration}$$

$$\boldsymbol{Dose\ index\ (DI)} = \frac{\boldsymbol{Actual\ cumulative\ dose}}{\boldsymbol{Planned\ cumulative\ dose}}$$

$$\boldsymbol{Time\ index\ (TI)} = \frac{\boldsymbol{Planned\ total\ duration^*}}{\boldsymbol{Actual\ total\ duration}}$$

*of actual treatment cycles

$$\boldsymbol{RDI} = \boldsymbol{DI} \times \boldsymbol{TI}$$

$$DI_{EOX} = \frac{DI_{epirubicin} + DI_{oxaliplatin} + DI_{capecitabine}}{3}$$

$$TI_{EOX} = \frac{TI_{epirubicin} + TI_{oxaliplatin} + TI_{capecitabine}}{3}$$

Treatment and assessments in the PALAESTRA trial

The study treatment in Paper IV consisted of external beam radiotherapy with 20 Gy in five fractions to the primary tumor followed by four cycles of systemic chemotherapy, Figure 13.

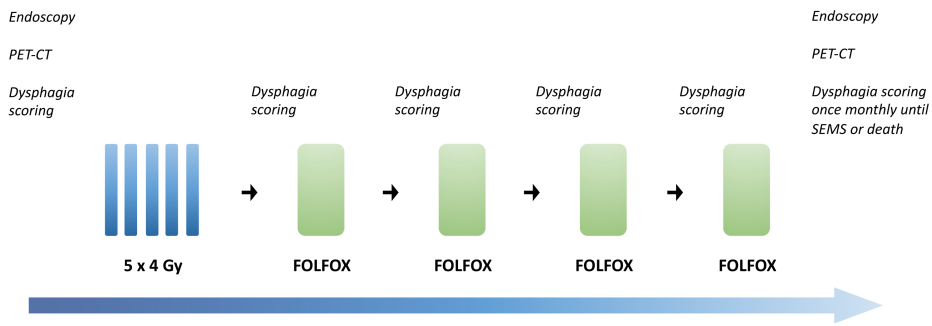

Figure 13.
Overview of the treatment and assessments in the PALAESTRA trial. SEMS = self-expanding metal stent.

Radiotherapy

An upper endoscopy, FDG-PET and CT were done within 3 weeks prior to treatment start to be used as baseline investigations and for radiotherapy dose-planning. It was at the discretion of the radiation oncologist to choose any of the following techniques:

- 3D-CRT (Three-dimensional conformal radiation therapy)
- IMRT (Intensity-modulated radiation therapy)
- VMAT (Volumetric-modulated arc therapy)
- HT (Helical tomotherapy)

The planned dose was 20 Gy delivered in five daily fractions, i.e. 4 Gy per fraction, with an overall treatment time of 5-8 days allowing for a gap during the weekend for patients not starting on a Monday.

Treatment volumes

- GTV (Gross Tumor Volume): esophageal primary tumor
- CTV (Clinical Target Volume): GTV + 5 mm radial margin (limited by pleuras, pericardium and vertebral bodies) and + 20 mm proximal and distal margin

- ITV (Internal Target Volume): CTV + 5 mm radial margin and + 10 mm cranio-caudal margin (could be smaller if 4D-CT was used)
- PTV (Planning Target Volume): ITV + set-up margin according to local routines
- OAR (Organs at Risk): spinal cord, lungs, heart, liver and kidneys
- PRV (Planning organ at Risk Volume): spinal cord + 5 mm radial margin
- In case of metastatic disease limited to adjacent local lymph nodes it was optional to include these in the GTV
- The study protocol also permitted additional separate targets, e.g. painful bone metastases, to be treated according to local routines

Organs at risk

Using conventional fractionation with 2 Gy per fraction, the maximum tolerated doses to organs at risk are:

- Spinal cord: 45 Gy point dose
- Lungs: 20 Gy to 30% of the lungs
- Heart: 50 Gy to 30% of the heart
- Liver: 30 Gy to 60% of the liver
- Kidneys: 17 Gy to 50% of the kidneys

Isoequivalent maximum tolerated doses using hypofractionation with 4 Gy per fraction were calculated using the linear-quadratic model [177], where D is the total dose, d is the dose per fraction and the α/β ratio is a measure of the fractionation sensitivity of a tissue:

$$\frac{D_2}{D_1} = \frac{d_1 + (\alpha/\beta)}{d_2 + (\alpha/\beta)}$$

assuming α/β ratios for late reactions:

- Spinal cord: α/β = 2 Gy
- Kidneys, heart, lungs and liver: α/β = 3 Gy

the maximum tolerated dose using 4 Gy per fraction are:

- Spinal cord: 30 Gy point dose
- Lungs: 14 Gy to 30% of the lungs

- Heart: 36 Gy to 30% of the heart
- Liver: 21 Gy to 60% of the liver
- Kidneys: 12 Gy to 50% of the kidneys.

Even though the total dose in each patient was only 20 Gy, it was emphasized to minimize the radiation dose to the organs at risk, Table 3.

Table 3.
Radiotherapy dose-volume restrictions (constraints) and recommendations (objectives) in PALAESTRA.

Priority	Volume	Objectives	Constraints
1	PTV		D99% ≥ 19 Gy D1% ≤ 21 Gy
2	Spinal cord	$D_{max} \leq 10$ Gy	
3	PRV Spinal cord	$D_{max} \leq 12$ Gy	
4	Lungs	$D_{mean} \leq 4$ Gy	
5	Heart	$D_{mean} \leq 10$ Gy	
6	Liver	$D_{mean} \leq 4$ Gy	
7	Kidneys	$D_{mean} \leq 2$ Gy	
8	Body	$D_{max} \leq 22$ Gy	

Chemotherapy

The protocol stated that the first cycle should start preferably 1-2 weeks after the last fraction of radiotherapy but could be postponed in case of severe toxicity. The planned chemotherapy was four cycles of FOLFOX (mFOLFOX6) but if the patient had parenteral nutrition occupying the central venous access and thus making a 44-h continuous fluorouracil infusion inconvenient, bolus administration of fluorouracil according to the Nordic FLOX regimen was allowed. It was not recommended to use granulocyte-colony stimulating factor (G-CSF). After end of the study treatment it was up to the treating physician to decide on further treatment.

FOLFOX*:*

- Fluorouracil 2400 mg/m^2, 44 hour infusion, day 1-3
- Fluorouracil 400 mg/m^2, bolus injection, day 1
- Calcium folinate 200 or 400 mg/m^2, day 1
- Oxaliplatin 85 mg/m^2, day 1

- Cycle length 14 days

FLOX*:*

- Fluorouracil 500 mg/m^2, bolus injection, day 1 and 2
- Calcium folinate 60 mg/m^2, day 1 and 2
- Oxaliplatin 85 mg/m^2, day 1
- Cycle length 14 days

Endpoints and assessments

The primary endpoint in PALAESTRA was improvement of dysphagia. Assessment of dysphagia was done by a study nurse, a treatment nurse or a physician, by phone or at patient visits to the clinic: at baseline; after radiotherapy; prior to each cycle of chemotherapy and then once every month during follow-up until SEMS-insertion or death. Scoring of dysphagia was based on the scale by Ogilvie [178], Table 4.

Table 4.
Scoring of dysphagia in PALAESTRA.

Score	Description
0	Able to eat a normal diet (no dysphagia)
1	Able to swallow some solid food
2	Able to swallow semi-solid food only
3	Able to swallow liquids only
4	Unable to swallow anything (complete dysphagia)

Secondary endpoints:

- Response of the primary tumor assessed using endoscopy
- Response of the primary tumor assessed using FDG-PET
- Response of the total disease burden assessed using FDG-PET
- Response of the total disease burden assessed using CT
- Overall survival
- Safety

Statistics

Differences in patient and clinicopathological factors grouped by PODXL expression (Paper I and II), DI or TI (Paper III) were assessed using chi-square test for categorical variables and Mann–Whitney *U* test or Kruskall-Wallis test for continuous variables. To assess differences in PODXL expression between tissue types (Paper I) we used Mann–Whitney *U* test. Differences in histopathologic response stratified by PODXL expression (Paper II) was assessed using chi-square test (linear-by-linear). We used Kendall's tau-b (τ) to assess correlation of PODXL expression between tissue samples (Paper II). Follow-up time was calculated with reverse Kaplan–Meier estimation. For time to recurrence (TTR) only a recurrence of the same cancer was defined as an event. For overall survival (OS) any death was defined as an event. Baseline dates were the date of the result of the diagnostic biopsy (Paper I), the resection date (Paper II), the date of the diagnostic biopsy (Paper III) or the date of enrollment (Paper IV). Survival was estimated using Kaplan–Meier and for comparison of the survival curves log-rank test was used. Hazard ratios (HR) for TTR and OS (Paper I and II) were derived from Cox proportional-hazards regression. An interaction term was used in the Cox regression analysis to assess whether PODXL expression was predictive for treatment benefit (Paper II). Odds ratios (OR) for histopathologic response vs. dose index and time index (Paper III) were calculated using binary logistic regression. All statistical tests were two-sided and a p-value < 0.05 was considered statistically significant.

In the PALAESTRA trial the following analysis populations were defined:

- The intention to treat (ITT) population included all patients registered for treatment within the study
- The safety population included all patients who received at least one fraction of radiotherapy
- The per protocol (PP) population included all patients who received a minimum of four fractions of radiotherapy and two cycles of protocol specified chemotherapy

Sample size calculation for the PALAESTRA study was based on a Simon's two-stage design [179] on the PP population testing the null hypothesis that the rate of dysphagia improvement was 50% against the alternative hypothesis that the rate was 75%. A response was defined as an improvement (from baseline) in dysphagia score by at least one step during the study treatment period or within four weeks after end of study treatment. In the first stage, 14 patients treated per protocol were to be included. If there were less than eight responders in these 14 patients, the study should stop enrollment. Otherwise the recruitment should continue to the second stage until a total of 23 patients treated per protocol were included. The null hypothesis would be rejected if there were 16 or more responders in these 23 patients. This design yielded a type I

error (α) rate of 0.05 and power (1- β) of 0.80 when the true response rate was 75% in the PP population. We assumed that 15% of the registered patients would not complete treatment per protocol why the estimated total sample size was 27 patients.

Summary of results and discussion

Paper I

In paper I it was shown that expression of PODXL in treatment-naîve, resected primary esophageal and gastric adenocarcimomas (cohort 1) was significantly higher in primary tumors and lymph node metastases compared to intestinal metaplasia ($p < 0.001$) and also significantly higher in intestinal metaplasia compared to adjacent normal epithelium ($p < 0.001$). This indicates that PODXL is involved in the initial tumorigenesis of gastric and esophageal adenocarcinoma and supports the suggested role of PODXL in epithelial-mesenchymal transition. We also showed that PODXL expression was associated with lymph node metastases ($p = 0.006$) and high grade (poorly differentiated) tumors ($p = 0.023$). Moreover it was shown that PODXL expression was an independent prognostic biomarker for reduced time to recurrence, HR 3.39 (95% CI 1.01-11.35) and poor overall survival, HR 2.03 (95% CI 1.04-3.98).

This was the first report on PODXL as a prognostic biomarker in esophageal adenocarcinoma and validated previous findings in gastric cancer [160].

Paper II

Patients with resectable esophageal or gastric adenocarcinoma treated with neoadjuvant chemotherapy (cohort 2) and having high PODXL expression (13% of the patients) in their pre-treatment biopsies had a remarkably good histopathologic response (36% with no residual cancer cells) and an excellent prognosis. There was no correlation between PODXL expression in pre-neoadjuvant biopsies and paired resected primary tumors and this might be explained by chemotherapy-induced alterations of PODXL expression or it could depend on intratumor heterogeneity.

In contrast to cohort 1 (Paper I), in which PODXL expression was a negative prognostic factor, PODXL expression was not a prognostic factor in cohort 2, suggesting that PODXL might be a predictive biomarker. In the pooled cohort there were no significant differences in time to recurrence or overall survival for patients with PODXL negative tumors who received neoadjuvant ± adjuvant chemotherapy

compared to surgery alone. In contrast, the PODXL positive cases treated with neoadjuvant ± adjuvant chemotherapy had a significantly longer time to recurrence and overall survival compared to those treated with surgery alone. For patients treated with neoadjuvant fluoropyrimidine and oxaliplatin ≥ 8 weeks ± adjuvant chemotherapy, a significant interaction term (PODXL expression x treatment) was shown in Cox regression in both unadjusted ($p = 0.006$) and adjusted ($p = 0.024$) analyses, further supporting a potential predictive role for PODXL. The interaction term was however not statistically significant for overall survival.

The suggested role of PODXL expression as a predictive biomarker for benefit of chemotherapy addition is in line with studies in colorectal [151] and periampullary [154] cancer in which only patients with high expression of PODXL in their resected tumors seemed to benefit from adjuvant chemotherapy.

Of note, the value of the adjuvant part of perioperative chemotherapy is unclear. In the MAGIC and FLOT4 trials only 55-66% of those who proceeded to surgery started the adjuvant part and of these 71-76% completed it. Retrospective studies on the importance of the adjuvant part are conflicting [180–182]. With this in mind we therefore chose to focus on the neoadjuvant part.

In summary, it is suggested that PODXL might be used as a predictive biomarker to select patients for either neoadjuvant ± adjuvant chemotherapy or surgery alone. However, this must be confirmed in additional studies. Moreover, improved biopsy sampling is advocated to maximize the chance of successful PODXL staining and to account for possible intratumor heterogeneity so that PODXL positive cases are not missed.

Paper III

Assessing the impact of dose reductions (DI) or treatment delays (TI) of neoadjuvant EOX in cohort 2, the only factor with a significant OR for a major response (0-10% residual cancer cells) was TI ≥ 0.95 with OR 8.40 (95% CI 1.02-69.37), whereas for a response with 0-50% residual cancer cells the only factor with a significant OR was DI ≥ 0.95 with OR 3.14 (95% CI 1.06-9.29). The small sample size (n=63) with few events precluded multivariable analyses, however there were no significant differences in patient or clinical characteristics between dichotomized groups of DI and TI at cutoff 0.95.

In Kaplan-Meier survival analyses both time to recurrence and overall survival were significantly improved in patients with a histopathologic response, with the largest difference noted with the cutoff at 10% residual cancer cells compared to the cutoff at 50%. Patients with 0-10% residual cancer cells had a 5-year overall survival of 87%

compared to 47% for patients with more than 10% residual cancer cells. For patients with 0-50% residual cancer cells the 5-year overall survival was 65% compared to 41% for patients with more than 50% residual cancer cells.

Regarding the clinical significance of histopathologic response, several studies have demonstrated an association with survival in esophageal and gastric adenocarcinoma, although with conflicting results whether it is an independent predictor [183, 184] or not [185–187].

The suggestion from this study, that avoiding treatment delays might be more important than maintaining full dose of chemotherapy, is in line with results from other studies on platinum-based treatment in metastatic colorectal cancer [176], resected ovarian cancer [188] and metastatic gastric cancer [189]. Given the limited sample size, precluding adjustment for other possible factors that might impact on histopathologic response (e.g. differentiation grade, tumor size and Laurén type [190, 191]), further studies are needed, preferably with larger patient cohorts.

Paper IV

In the PALAESTRA trial a total of 29 patients were recruited of whom 23 were treated in accordance with the per protocol definition. The overall rate of dysphagia improvement was 79%, the median duration of improvement was 6.7 months for all patients and 12.2 months for the responders. The median overall survival for all patients was 9.9 months. Only five patients received SEMS and none received additional radiotherapy to the primary tumor. In the per protocol population the rate of dysphagia improvement was 91% (thus the trial met the primary endpoint), the median duration of improvement was 12.2 months for all patients and 14.0 months for the responders. The median overall survival in the per protocol population was 16.0 months. Toxicities were manageable and the most common grade 3-4 adverse events were neutropenia (29%), infection (25%), anorexia (11%), esophagitis (11%) and fatigue (11%).

To the best of our knowledge, the rate and duration of dysphagia improvement in PALAESTRA compares favorably to previous trials, e.g. the recent phase III trial TROG 03.01 [127] in which patients with incurable esophageal cancer (~ 70% adenocarcinomas) were randomized to palliative chemoradiotherapy (30 or 35 Gy with concomitant fluorouracil and cisplatin) or radiotherapy alone (30 or 35 Gy). In TROG 03.01 the rate of dysphagia improvement sustained for at least four weeks was 45% and 35%, the rate of improvement at any assessment was 62% and 53% and the duration of improvement was 3.4 and 2.5 months for chemoradiotherapy vs. radiotherapy.

As far as we know, the treatment sequence in PALAESTRA as well as the use of external beam radiotherapy with 20 Gy in five fractions have not been previously studied in a prospective trial in esophageal cancer.

Given the demonstrated efficacy and manageable toxicities, the PALAESTRA concept is appealing for patients in good performance status and where the burden of metastatic disease does not necessitate immediate start of chemotherapy. However, due to the limited size of the study population and the non-randomized setup, additional prospective studies are warranted to confirm this.

Conclusions and future perspectives

Based on the results herein, it is suggested that:

- For patients with esophageal or gastric adenocarcinoma treated with surgery up-front, PODXL expression is an independent prognostic biomarker for reduced time to recurrence and short overall survival
- For patients with esophageal or gastric adenocarcinoma treated with neoadjuvant ± adjuvant chemotherapy, PODXL expression in pre-treatment biopsies is an independent predictive biomarker for benefit of chemotherapy
- Treatment delays of neoadjuvant chemotherapy in esophageal or gastric adenocarcinoma should be avoided in order to achieve a major histopathologic response
- Initial palliative short-course radiotherapy with 20 Gy in five fractions followed by chemotherapy is a promising new treatment strategy that can provide long-lasting relief of dysphagia in patients with esophageal adenocarcinoma

For PODXL it would be of interest to investigate whether there are any differences in its expression between the TCGA molecular subtypes [42, 43]. Moreover, the proposed role of PODXL as a predictive biomarker is of greater importance than its prognostic role since the former actually could have a direct impact on treatment decisions. To validate PODXL as a predictive biomarker it should ideally be explored (post hoc) in randomized trials with surgery alone in the control arm, e.g. MAGIC [65] and FFCD 9703 [67] for perioperative chemotherapy and ACTS-GC [73] and CLASSIC [74] for adjuvant chemotherapy.

The possible differential impact of chemotherapy dose reductions and treatment delays is of importance since it could have clinical implications for patients struggling with toxicities. This merits further investigations, not only in esophageal and gastric adenocarcinoma treated with neoadjuvant chemotherapy, but also in the palliative

treatment setting, in other malignancies, with other regimens and with different endpoints.

Regarding the PALAESTRA trial we will do an in-depth analysis of the radiotherapy part, e.g. comparing the different radiotherapy techniques used vs. fulfilment of dose-volume objectives and constraints.

To continue exploring the PALAESTRA concept the next step could be a randomized phase II/III trial with chemotherapy up-front in the control arm. Another possibility could be to address the issue that chemotherapy is delayed for a month with the current concept and therefore, in a phase I trial, try to find a safe dose level of chemotherapy to deliver concomitantly with 5 x 4 Gy. In addition, even though esophageal adenocarcinoma and esophageal squamous cell carcinoma are biologically different diseases, given the high radiosensitivity with conventional fractionation to the latter subtype, patients with squamous cell carcinoma might also be included in these trials.

It would also be of interest to explore the PALAESTRA concept as a neoadjuvant treatment in resectable esophageal cancer, possibly with FLOT instead of FOLFOX. Such an approach would be analogous to the experimental arm in the soon to be reported RAPIDO trial [192] in locally advanced rectal cancer where standard chemoradiotherapy followed by resection, with or without adjuvant chemotherapy, is compared to short-course radiotherapy (5 x 5 Gy) followed by chemotherapy (CAPOX) and resection without adjuvant treatment.

Another intriguing question is whether the PALAESTRA concept, or 5 x 4 Gy alone, in esophageal cancer, can render the irradiated primary tumor more immunogenic and thus increase the chance of a systemic tumor response when combined with immunotherapy, possibly via the abscopal effect [193, 194]. An initial approach could be to, in a prospective trial, sample biopsies (tissue and liquid) before and after short-course radiotherapy (and after chemotherapy, if any) for analysis of possible changes in immune signatures.

Populärvetenskaplig sammanfattning
(summary in Swedish)

Matstrups- och magsäckscancer utgör tillsammans den fjärde vanligaste cancerformen i världen med ca 1,6 miljoner nya fall per år och är den näst vanligaste orsaken till cancerrelaterad död. För hundra år sedan var magsäckscancer den cancerform som drabbade flest människor i världen men de senaste femtio åren har antalet som insjuknar stadigt minskat. Det finns två huvudsakliga typer av matstrupscancer där skivepitelcancer är den sort som dominerar globalt men i många västländer har insjuknandet i den andra sorten, adenocarcinom, ökat dramatiskt de senaste decennierna, bl a i Sverige där det numera är den vanligaste sorten. I Sverige är dessa sjukdomar inte så vanliga men 2013-2017 insjuknade varje år ca 1200 personer i matstrups- eller magsäckscancer, varav knappt 500 i magsäckscancer, ca 500 i matstrupscancer av adenocarcinomtyp och ca 200 i matstrupscancer av skivepiteltyp.

Som med de flesta cancerformer hos vuxna är ålder och rökning riskfaktorer för att drabbas av matstrups- eller magsäckscancer som dessutom är vanligare hos män. Intag av frukt och grönt verkar däremot minska risken att insjukna. Den dominerande riskfaktorn för magsäckscancer är "magsårsbakterien" *Helicobacter pylori*. För adenocarcinom i matstrupen är sura uppstötningar och övervikt riskfaktorer medan skivepitelcancer utöver rökning även kan ha ett samband med hög alkoholkonsumtion.

För att diagnostisera matstrups- eller magsäckscancer görs en gastroskopi där man även tar biopiser (vävnadsprover) från tumören och därefter görs en skiktröntgen för att se om tumören är lokaliserad eller har hunnit sprida sig till andra organ. För patienter med lokaliserad sjukdom kan kirurgi möjliggöra en chans till bot men endast cirka 20-25% lever efter fem år. Att till kirurgi komplettera med onkologisk behandling med cytostatika, med eller utan strålbehandling, har visat sig kunna öka andelen långtidsöverlevare. En standardbehandling vid magsäckscancer och matstrupscancer av adenocarcinomtyp är ett ge cytostatika före och efter operation och med denna strategi kan andelen som lever efter fem år öka till uppemot 45%. För de patienter som har spridning till andra organ eller som inte bedöms tåla kirurgi får man i regel inrikta sig på palliativ behandling med syfte att lindra symtom, förbättra livskvaliteten och förlänga livet. För palliativa patienter är prognosen dyster med en medianöverlevnad mindre än ett år även med onkologisk behandling.

Matstrups- och magsäckscancer är således vanliga sjukdomar globalt sett och med dålig prognos varför vi behöver lära oss mer om dessa sjukdomar för att kunna bli bättre på att behandla dem. I mitt avhandlingsarbete som består av fyra delarbeten har jag fokuserat på adenocarcinom i matstrupe och magsäck, dels för att dessa är de vanligaste formerna av matstrups- och magsäckscancer i Sverige och dels för att de liknar varandra mer än skivepitelcancer i matstrupen.

PODXL (Podocalyxin-like protein 1) är ett protein i cellens ytskikt som reglerar sammanhållandet av celler, bl.a. i njurar där det reglerar filtreringen av urin och i blodkärl. Det har också visats att överuttryck av PODXL i olika tumörformer ofta är förknippat med aggressiv sjukdom och dålig prognos.

I delarbete I har vi på sparat vävnadsmaterial från en grupp av 174 patienter som 2006-2010 opererats (utan onkologisk förbehandling) för adenocarcinom i matstrupe eller magsäck undersökt uttrycket av PODXL. Vi visade att uttrycket av PODXL var högre i cancerceller än i normala celler och var förknippat med spridning till lymfkörtlar och aggressivt tumörcellsutseende. Dessutom visade vi att de patienter som hade uttryck av PODXL i den bortopererade tumören hade sämre överlevnad.

I delarbete II har vi också undersökt PODXL men denna gång i vävnadsmaterial från 148 patienter med adenocarcinom i matstrupe eller magsäck som 2008-2014 fick förbehandling med cytostatika före operation. Här såg vi att tumörer med högt uttryck av PODXL, i de biopsier som tagits vid diagnos, var de som efter cytostatikabehandling och operation var de som hade minst andel kvarvarande cancerceller. I vidare analyser där vi även inkluderade patientgruppen från delarbete I visade det sig att patienter med PODXL-positiva tumörer verkade vara den grupp som hade nytta av förbehandling med cytostatika medan det för de med PODXL-negativa tumörer inte var någon överlevnadsskillnad om de bara opererades eller om de även fick tillägg med cytostatika. Sammanfattningsvis talar våra resultat för att PODXL i diagnostiska biopsier skulle kunna användas för att identifiera de patienter som kan ha nytta av förbehandling med cytostatika så att resten kan besparas det och behandlas med enbart kirurgi. Detta behöver dock bekräftas i ytterligare studier.

Ett vanligt bekymmer vid cytostatikabehandling före (och efter) operation av matstrups- eller magsäckscancer är att patienterna är sköra och har svårt att tåla behandlingen. Det är därför vanligt att man måste reducera cytostatikadoserna eller skjuta upp behandlingarna men vad detta har för påverkan på behandlingseffekten är väldigt lite studerat. Vi har därför i delarbete III undersökt just detta på en grupp med 63 patienter från delarbete II som alla förbehandlades med samma typ av cytostatikaregim och därefter genomgick operation. Vi tittade på andelen kvarvarande cancerceller i de bortopererade tumörerna som förbehandlats med cytostatika och

undersökte samband mellan dosreduktion respektive behandlingsuppskjutning. Vi fann ett samband mellan 0-10% kvarvarande cancerceller och att ge behandling i tid och ett samband mellan 0-50% kvarvarande cancerceller och att upprätthålla doserna. Vidare påvisades ett samband mellan andelen kvarvarande cancerceller och överlevnad med bäst prognos för de med endast 0-10% kvarvarande cancerceller. Sammantaget talar detta för att det kan vara viktigare att ge behandlingarna i tid än att ge fulla doser. Tyvärr var det för få patienter i studien för att vi skulle kunna väga in andra eventuella faktorer i analyserna så resultaten måste tolkas med försiktighet och bekräftas i fler studier.

Majoriteten av patienterna med matstrupscancer lider av dysfagi (sväljningssvårigheter) vilket medför nutritionsproblem, viktnedgång och dålig livskvalitet. I delarbete IV har vi i Lund genomfört en klinisk studie, benämnd PALAESTRA, där patienter med dysfagi pga obotligt adenocarcinom i matstrupen behandlades med hypofraktionerad strålbehandling följt av cytostatika. Hypofraktionering innebär en högre stråldos per behandling men färre behandlingstillfällen. I PALAESTRA-studien fick patienterna fem strålbehandlingar med dosen 4 Gray och därefter fyra cytostatikabehandlingar. Syftet var att få en god och långvarig förbättring av sväljningsförmågan. Mellan oktober 2014 och maj 2018 inkluderades totalt 29 patienter i studien. Av dessa var det 79% som fick förbättring av dysfagi (de flesta så att de kunde äta all typ av fast föda) och i median varade förbättringen i ungefär ett år vilket är bättre än i någon tidigare studie. Biverkningarna var hanterbara och de allvarligaste var låga nivåer av vita blodkroppar (29%), infektion (25%), viktnedgång (11%), irritation i matstrupen (11%) och trötthet/utmattning (11%). Sammantaget bedöms denna nya behandlingsstrategi som mycket lovande med stor chans till god och långvarig förbättring av dysfagi. Vi planerar att undersöka detta vidare i ytterligare kliniska studier.

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